Inhibition of Sympathetic Vasoconstriction Is a Major Principle of Vasodilation by Nitric Oxide In Vivo

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Abstract The objective of this study was to determine whether vasodilator effects of nitric oxide (NO) can be explained by the inhibition of vasoconstriction caused by peripheral sympathetic nerve activity (SNA) in vivo. For this purpose, we studied the effects of systemic inhibition of NO synthesis during experimental variation of SNA in anesthetized cats. Intravenous infusion of N\textsuperscript{G},nitro-L-arginine methyl ester (L-NAME, 10 mg/kg) in baroreceptor-intact animals (n=6) caused increases in mean arterial blood pressure (MAP) from 105.8±3.4 to 192.0±4.3 mm Hg that were associated with slight decreases in preganglionic SNA recorded from the white ramus of the third thoracic segment. Higher SNA appeared in completely baroreceptor-denervated cats (n=10) than in the intact cats, but no changes in nerve activity occurred after the subsequent administration of L-NAME. In contrast, MAP increased from 123.3±4.0 to 245.8±5.1 mm Hg. In baroreceptor-denervated cats, reversible suppression of peripheral SNA produced by cooling of the ventral surface of the rostral ventrolateral medulla oblongata (RVLM) caused significant hypotension (61.1±2.6 mm Hg) and almost completely reversed the hypertension caused by L-NAME (76.0±3.7 mm Hg). Intravenous administration of the α\textsubscript{2}-adrenergic receptor antagonist prazosin after L-NAME reduced MAP to a similar extent. In contrast, hypertension induced by angiotensin II could not be reversed by RVLM cooling. The pressor effects of intravenously administered noradrenaline during RVLM cooling were markedly potentiated by L-NAME and attenuated by the NO-donor compound S-nitroso-N-acetylpenicillamine (SNAP). These results demonstrate that inhibition of peripheral sympathetic vasoconstriction is an important mechanism of vasodilation by NO in vivo and suggest that the vascular effects of NO may be very closely linked to the regulation of cardiovascular functions by the central nervous system. (Circ Res. 1994;75:1073-1077.)

Key Words • nitric oxide • neurogenic vasoconstriction • sympathetic nerve activity • N\textsuperscript{G},nitro-L-arginine methyl ester • cats

The vascular release of nitric oxide (NO), which is probably identical with the endothelium-derived relaxing factor (EDRF),\textsuperscript{1,2} causes tonic reduction of smooth muscle tone in vivo.\textsuperscript{3} Its synthesis is inhibited by guanidino-substituted L-arginine analogues, such as N\textsuperscript{G},nitro-L-arginine (L-NNA), N\textsuperscript{G},nitro-L-arginine methyl ester (L-NAME), or N\textsuperscript{G}-monomethyl-L-arginine.\textsuperscript{4} Systemic administration of these inhibitors in anesthetized and conscious animals causes marked blood pressure increases and impairment of regional circulation.\textsuperscript{5,6} Apart from the direct actions of NO on vascular smooth muscle, additional roles for NO in the central nervous regulation of cardiovascular functions have been proposed. Several groups have shown that systemic inhibition of the synthesis of NO caused changes in peripheral sympathetic nerve activity (SNA) in rats\textsuperscript{7-11} and in rabbits,\textsuperscript{12} effects that were, however, influenced by concomitant changes in the baroreflex control of SNA. At sympathetic nerve endings, another important effect of NO may occur. Recent in vitro studies revealed inhibitory effects of NO and potentiating effects of L-NNA\textsuperscript{13,14} or L-NAME\textsuperscript{15,16} on the vasoconstrictor responses to electrical sympathetic nerve stimulation, suggesting a peripheral modulation of the sympathetic vasoconstriction by NO that is independent of the degree of central SNA. Finally, the involvement of putative nitroergic peripheral vascular nerves has been recently discussed, since in pentobarbital-anesthetized dogs pharmacological ganglionic blockade prevented hypertension induced by inhibition of NO synthesis, whereas the broad spectrum adrenergic antagonist phentolamine was much less effective.\textsuperscript{17}

The aim of the present study was to test the hypothesis that the vasodilator actions of vascular NO in vivo can be explained, at least in part, by peripheral inhibition of sympathetic vasoconstriction. For this purpose, choralose-anesthetized cats were prepared for reversible cooling of the rostral ventrolateral medulla oblongata (RVLM), the area within the brainstem that controls central sympathetic outflow.\textsuperscript{18} After complete baroreceptor denervation, we studied the effects of a substantial reduction in central SNA by RVLM cooling before and after inhibition of NO synthesis by L-NAME and compared it with the effects of angiotensin II and α\textsubscript{2}-receptor blockade by prazosin. In addition, dose-response curves for intravenously infused noradrenaline during RVLM cooling were performed before and after the administration of L-NAME as well as after the administration of the spontaneously NO-releasing compound S-nitroso-N-acetylpenicillamine (SNAP).

Materials and Methods
Experiments were performed on cats (2.5 to 4.5 kg body weight) that were initially sedated by ketamine (10 mg/kg IM) and anesthetized by α-chloralose (40 to 50 mg/kg IV). Addi-

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tional doses of α-chloralose were administered when required. Catheters were placed into a femoral vein and artery for infusion of drugs and for measurement of arterial blood pressure via a pressure transducer (Statham PD231D), respectively. The cats were paralyzed by 0.6 mg pancuronium bromide and artificially ventilated via a tracheal cannula. Rectal temperature was maintained at 38.0°C to 38.5°C by a thermostatically controlled infrared lamp.

For complete baroreceptor denervation, the carotid sinus and vagoaortic nerves were cut. The denervation was verified by the absence of changes in SNA during blood pressure increases caused by 1 μg/kg IV noradrenaline. The left white ramus (n=16) of the third thoracic segment (WR-T3) was exposed retroperitoneally after removal of the head and dorsal portion of the second and third ribs. The renal nerve (n=4) was exposed retroperitoneally. To minimize thoracic movements due to ventilation, a procedure producing a pneumothorax was performed. All nerves were kept in a pool of warm paraffin oil and placed on bipolar electrodes for SNA recording. Neural signals were amplified and filtered (model AM 502 amplifier; bandwidth, 10 Hz to 10 kHz; Tektronix). WR-T3 activity was recorded as counted impulses at intervals of 0.3 second and is expressed in the figures as impulses per second, whereas renal nerve activity is presented as a resistance-capacity-integrated signal. For SNA inhibition by cooling, the ventral surface of the RVLM was exposed. In the cat, the RVLM projects onto the ventral surface 1 mm rostral to the most cranial rootlet of the 12th cranial nerve and 4 mm lateral to the midline. Cooling of the RVLM was performed by using a hollow metal thermode shaped to closely contact both sides of the RVLM simultaneously. The temperature of the inner surface of the thermode was continuously monitored and during control conditions was kept at 38.5°C by perfusion of thermostatically controlled warm water. For cooling the RVLM to 5.0°C to 3.0°C, the perfusion of the thermode was switched to cold ethanol provided by a cryostat. The desired temperature level could be maintained by manually adjusting the flow through a clamp. To prevent irreversible damage of neuronal structures, the duration of cooling was limited to 4 to 8 minutes.

L-NAME, prazosin (Sigma), and SNAP (Schwarz Pharma) were dissolved in Ringer’s solution. Noradrenaline (norepinephrine [Aterenol], Hoechst) and angiotensin II (Hypertensin, Ciba-Geigy) were obtained as ready-for-use solutions. All drugs were further diluted in Ringer’s solution and administered by short-term infusion at the doses stated in “Results.” Measurements were taken when steady-state responses were reached.

Data were analyzed by ANOVA for repeated measurements, and comparison of means was carried out by the Tukey method. For comparison of means between groups of different animals (intact and denervated), t tests with the Bonferroni correction were used (SAS Institute). Differences of P<.05 were considered to be significant. Values are reported as mean±SEM.

Results

Effects of Systemic NO Blockade in Intact and Baroreceptor-Denervated Cats

Changes in mean arterial pressure (MAP) and preganglionic SNA (WR-T3) in response to systemic inhibition of NO synthesis by intravenous infusion of L-NAME (10 mg/kg) in intact (n=6) and baroreceptor-denervated (n=10) cats are shown in Fig 1. In intact cats, L-NAME increased MAP, leading to decreases in SNA, which, however, did not reach statistical significance. In baroreceptor-denervated cats (n=10), mean baseline SNA and MAP were higher, but the administration of L-NAME did not significantly alter SNA during the whole study period in these animals. However, the pressor responses to L-NAME were significantly enhanced when compared with the intact group (P<.01, t test). When baroreceptor denervation was performed in intact animals after the L-NAME treatment (n=4), MAP and SNA values appeared that were similar to those observed in animals that had been denervated before the administration of L-NAME (data not shown). This unmasking of pressor responses to L-NAME after removal of the baroreflex inhibition of SNA is shown in Fig 2. This figure also shows the closely parallel courses of preganglionic SNA (WR-T3) and postganglionic SNA (renal nerve), which were recorded simultaneously in four experiments. Neither RVLM cooling nor any drug used in the present study altered the relation between these nerve activities.

Effects of RVLM Cooling

The effects of experimental variation of central sympathetic tone on the peripheral vasoconstrictor effects of L-NAME and angiotensin II were studied in baroreceptor-denervated cats (n=10). The obligatory role of the presence of SNA for the pressor effects of NO blockade is shown in a representative tracing in Fig 3. Cooling of the RVLM almost completely reversed the hypertension induced by L-NAME. This was likewise achieved by α1-adrenergic receptor blockade during NO blockade (Fig 3B). Additional depressor effects of RVLM cooling thereafter may be related to reductions in cardiac output caused by bradycardia and the lack of inotropic sympathetic effects. Fig 4 summarizes the depressor effects of effective inhibition of peripheral SNA by RVLM cooling. RVLM cooling reduced MAP
in L-NAME–treated denervated cats from 245.8±4.9 to 76.0±3.7 mm Hg, which represents a mean difference of 169.8 mm Hg. In contrast, after induction of hypertension by angiotensin II (1.5 μg/kg per minute), RVLM cooling caused only a mean decrease of 63.7 mm Hg, which is similar to the hypotensive responses during control conditions. As shown by the concomitant changes in preganglionic SNA in Fig 4, RVLM cooling effectively suppressed SNA irrespective of the different infusions. At the concentrations used in the present study, none of the applied substances significantly altered central sympathetic tone.

**NO Dependence of the Responses to Intravenous Noradrenaline**

Presynaptic or postsynaptic mechanisms or both could mediate peripheral inhibition of sympathetic vasoconstriction. The involvement of presynaptic mechanisms is shown in Fig 5. We assessed dose-response relations for intravenously infused noradrenaline (upper panel) in baroreceptor-denervated cats (n=10) during RVLM cooling to suppress the neural constrictor tone. The average preganglionic SNA throughout these measurements was 48.5±9 impulses per second. The vascular sensitivity to the pressor effects of noradrenaline was significantly enhanced by L-NAME. The opposite effect, ie, marked attenuation of the pressor responses to noradrenaline, was observed when dose-response relations were obtained after the administration of the spontaneously NO-releasing compound SNAP (60 μg/kg) in L-NAME–treated cats (n=5). Exogenous NO supply decreased the responses to noradrenaline in these animals more than 10-fold. In the lower panel of Fig 5, similar dose-response relations for angiotensin II, obtained from four cats, are depicted. The modulation of the dose-response relation for angiotensin II by the absence (L-NAME) or presence

**Fig 2.** Representative tracings showing unmasking of the effects of Nω-nitro-L-arginine methyl ester (L-NAME) after baroreceptor denervation. Preganglionic (WR-T3) and postsynaptic (fRN) sympathetic nerve activity, blood pressure (BP), and heart rate (HR) were recorded simultaneously. Neural activity was amplified and recorded as a resistance-capacity-integrated signal (fRN). L-NAME (10 mg/kg IV) was administered 10 minutes before the middle tracings were recorded. Baroreceptor denervation was performed within 5 minutes. bpm indicates beats per minute; imp/s, impulses per second; and AU, arbitrary units.

**Fig 3.** Tracings showing the effects of inhibition of nitric oxide synthesis and rostral ventrolateral medulla oblongata (RVLM) cooling (A) or intravenous administration of prazosin (300 μg/kg) (B) in a baroreceptor-denervated cat. Note that the severe hypertension induced by Nω-nitro-L-arginine methyl ester (L-NAME, 10 mg/kg) is completely reversed by either inhibition of sympathetic nerve activity (SNA) or α1-adrenergic receptor blockade. Preganglionic SNA (WR-T3), arterial blood pressure (BP), and heart rate (HR) were recorded. bpm indicates beats per minute; imp/s, impulses per second.

**Fig 4.** Bar graphs showing changes in mean arterial blood pressure (MAP) and preganglionic sympathetic nerve activity (WR-T3) in response to Nω-nitro-L-arginine methyl ester (L-NAME), angiotensin II (All), and prazosin (after L-NAME) in baroreceptor-denervated cats (n=10) during control conditions and after suppression of sympathetic outflow by rostral ventrolateral medulla oblongata (RVLM) cooling. L-NAME and prazosin were administered at the doses stated in Fig 3; All was infused at 1.5 μg·kg⁻¹·min⁻¹ IV. Measurements during RVLM cooling were taken when steady-state conditions had been reached (~2 minutes). **P<.01 (mean±SEM). imp/s indicates impulses per second.
Similar results have been obtained in rats.\(^9\) The present study has revealed peripheral inhibitory effects of NO on sympathetic vasoconstriction that are independent of the central sympathetic tone, since baseline SNA was not altered by L-NAME in the baroreceptor-dener-
ated cats used for the RVLM cooling experiments. The effects of \(\alpha\)-adrenergic receptor blockade support this view. Furthermore, previous in vitro studies revealed an attenuation of the constrictor effects of electrical symp-
athetic nerve stimulation by endothelial NO,\(^13,19,20\) which is in line with our observations.

By use of pharmacologic ganglionic blockade, similar reductions of the pressor responses to NO blockade have been recently demonstrated in dogs\(^16\); these reductions were interpreted by this group as a result of inhibition of putative nitroxidergic nerves mediating tonic relaxation, since phentolamine was much less effective. In another study on rats,\(^10\) hexamethonium failed to reverse the rather small hypertensive effects (+22 mm Hg), which were evoked by L-NAME in these animals, but in accordance with our findings (Fig 5), marked potentiation of pressor responses to phenyleph-
rine by L-NAME were detected, whereas increases in pressor responses to angiotensin II after the administration of L-NAME were much smaller. Our experi-
ments show that transient SNA inhibition is associated with a reversible and nearly complete disappearance of the pressor effects of L-NAME, suggesting that NO directly inhibits sympathetic vasoconstriction. The rela-
tion between preganglionic (WR-T3) and postgangli-
onic (renal nerve) SNA, which was recorded simultane-
ously in four experiments (Fig 2), was neither affected by NO blockade nor by any other treatment. Significant effects of L-NAME on ganglionic transmission can therefore be ruled out. The observed effects of NO blockade are thus probably related to direct actions of NO at the nerve endings on smooth muscle cells. The origin of such adventitial NO remains to be determined. However, endothelial NO probably can reach the ad-
ventitial side at least in resistance vessels.\(^3,4,21\) Furthermore, it is possible that neuronally released NO may contribute to this effect, as recently suggested by Toda et al.\(^6\) This hypothesis has been supported by histo-
chemical studies in the dog, revealing NO synthase immunoreactive nerve fibers in the adventitia of differ-
ent vessels.\(^22\)

It has been shown in other species that systemic inhibition of NO synthesis can produce increases in baseline SNA,\(^9,11,12\) especially when high doses of the L-arginine analogues are used. Therefore, it is possible that NO has a dual function in the sympathetic nervous system in vivo, comprising both a reduction of central sympathetic tone and peripheral inhibition of its con-
strictor effects. There are studies that support this hypothesis. In rats chronically treated with L-NAME compared with control rats, much greater decreases in arterial blood pressure occurred after ganglionic blockade,\(^23\) but acute administration of L-NAME induced a potentiation of sympathetic vasoconstriction evoked by electrical nerve stimulation in pithed rats also.\(^15\)

Additional muscarinic blocking activity, which poten-
tially could have produced side effects, has been re-
ported for L-NAME.\(^24\) In cats, however, no differences exist between the effects of L-NNA and L-NAME.\(^25\) Furthermore, in all experiments on baroreceptor-dener-

**Fig 5.** Graphs showing the nitric oxide (NO) dependence of the dose-response relations for intravenously administered nor-
adrenaline (upper panel, \(n=10\)) and angiotensin II (lower panel, \(n=4\)) during rostral ventrolateral medulla oblongata (RVLM) cooling. Inhibition of NO synthesis was performed after control experiments by intravenous administration of N\(^5\)-nitro-L-arginine methyl ester (L-NAME, 10 mg/kg; \(n=10\)). Exogenous NO was supplied by \(S\)-nitroso-N-acetylcysteine (SNAP, 60 \(\mu\)g/kg IV, for noradrenaline, \(n=5\)) after the administration of L-NAME. Noradrenaline and angiotensin II were administered by consecu-
tive bolus injections of single (increasing) doses during short periods (2 to 3 minutes) of RVLM cooling, which were followed by recovery periods of 3 to 5 minutes. **\(P<.01\) (mean±SEM).

(SNAP) of NO was directionally similar but much weaker when compared with the noradrenaline dose-response relation, suggesting a specific interaction between NO and the vasoconstrictor mechanisms for noradrenaline.

**Discussion**

This is the first study that describes the direct relation between the pressor effects of systemic inhibition of NO and the peripheral sympathetic vasoconstrictor tone in vivo. By nonpharmacologic adjustment of SNA, avoiding ganglionic blockade by drugs, we were able to demonstrate that the effects of inhibition of SNA are fully reversible. \(\alpha\)-Chloralose-anesthetized and unre-
strained conscious cats have comparable baseline SNA,\(^18\) Therefore, at least in the cat, vasodilation caused by vascular release of NO may be explained through its inhibitory effects on sympathetic vasocon-
striction. The observed effects of NO blockade on SNA in baroreceptor-intact animals (SNA reduction) and the responses to baroreceptor denervation (SNA increases) are directly related to the integrity of the baroreflex.
vated cats (vagotomy), subsequent muscarinic blockade would not have been relevant.

Both presynaptic and postsynaptic mechanisms could account for the observed phenomenon. The leftward shift by L-NAME of the dose-response relations for noradrenaline in the absence of significant SNA and the analogous rightward shift caused by exogenous NO (Fig 5) provide evidence that postsynaptic mechanisms may be important. This evidence is supported by in vitro studies in which L-NAME did not alter noradrenaline release from sympathetic nerve endings. Interestingly, the endothelium was a prerequisite for the potentiation of vasoconstrictor effects of noradrenaline by L-NAME in these experiments. Possible additional presynaptic effects of NO on the release of noradrenaline from sympathetic nerves have been recently suggested by others. Vasodilation by NO may involve specific actions on α-adrenergic constrictor mechanisms, since pressor responses to angiotensin II were directionally similar but much less affected by L-NAME or SNAP when compared with noradrenaline responses (Fig 5). However, the site and type of this interaction remain to be elucidated. Finally, sympathetic constrictor can, on the other hand, enhance vascular NO release by increasing endothelial shear stress and by direct agonistic stimulation, as previously shown in pharmacologic studies using α-adrenergic agonists. Thus, the neurogenic control of vasomotor tone may be locally adjusted by complex feedback mechanisms.

In summary, the present study suggests that the vasodilator effects of NO are more closely linked to the central regulation of cardiovascular functions by the autonomic nervous system than hitherto believed. Sympathetic control of vascular resistance in humans is even greater than in cats. Deficient counterbalance of sympathetic influences may thus be an important factor in the pathogenesis of the variety of cardiovascular diseases that are associated with reduced synthesis of vascular NO.

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